



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2004

Protease-activated receptors and EDHF: the icing on the cake?

Traupe, T ; Barton, M

DOI: <https://doi.org/10.1016/j.cardiores.2004.01.008>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-153852>

Journal Article

Published Version

Originally published at:

Traupe, T; Barton, M (2004). Protease-activated receptors and EDHF: the icing on the cake? Cardio-vascular Research, 61(4):645-647.

DOI: <https://doi.org/10.1016/j.cardiores.2004.01.008>

Editorial

Protease-activated receptors and EDHF: the icing on the cake?

Tobias Traupe, Matthias Barton*

Medizinische Poliklinik, Departement für Innere Medizin, Universitätsspital Zürich, Rämistrasse 100, CH-8091 Zürich, Switzerland

Received 29 December 2003; accepted 7 January 2004

See article by Kawabata et al. (pages 683–692) in this issue.

The regulation of vascular smooth muscle cell tone by the vascular endothelium was initially described by Furchgott and Zawadzki, suggesting the existence of an endothelium-derived relaxing factor (EDRF) acting through cGMP, which was later identified as nitric oxide (NO) (see reference [1] for review). Since then, other actions of NO such as control of cell growth, blood cell–endothelial cell interactions, immunomodulatory functions, and anti-aggregatory effects have been described [2]. In addition to NO and cyclooxygenase products, a third endothelium-derived principle, namely hyperpolarization of vascular smooth muscle cells in response to an endothelium-derived hyperpolarizing factor (EDHF), has been identified [3]. Production and bioactivity of endothelial factors are disturbed in cardiovascular diseases, and endothelium-dependent dilator mechanisms are counteracted by vasoconstrictory prostanoids that contribute to abnormal vascular function [4].

In this issue of *Cardiovascular Research*, Kawabata et al. report that activating peptides of the protease-activated receptor 2 (PAR2), namely trypsin and SLIGRL, cause endothelium-dependent relaxation by activating NO and EDHF pathways in rat mesenteric arteries in vitro. The authors show that gap junctions, but not epoxyeicosatrienoic acids, hydrogen peroxide, or K^+ are involved in the release of EDHF in response to activation of PAR2. Moreover, vascular contractions due to an activating peptide of the protease-activated receptor 1 (TFLLR) were observed after inhibition of EDHF and NO or after endothelium removal.

1. Vascular expression and function of protease-activated receptors

The protease thrombin is generated during activation of coagulation and converts fibrinogen to soluble fibrin [5]. In addition, thrombin signals directly to endothelial cells, vascular smooth muscle cells (VSMCs), platelets, fibroblasts and other cell types by cleaving protease-activated receptors (PARs), which belong to the G-protein-coupled receptor family [6]. The discovery of the first PAR, namely PAR1, provided a new paradigm for receptor activation [7]. Since then, three other PARs have been cloned and shown to play a role in cellular responses to vascular injury, inflammation and wound healing. Thrombin acts as an agonist on PAR1, PAR3, and PAR4 with different potencies [6] whereas PAR2 is activated by both trypsin and the thrombin-cleaved PAR1. Moreover, several thrombin receptor-activating peptides (TRAPs) also activate PARs [6]. The binding of a PAR agonist to the receptor is irreversible once the receptor is cleaved, and it is therefore rather unlikely that these pathways are “routinely” involved in cellular signaling, particularly since the level of expression widely differs in various vascular cell types. Interestingly, PARs are not only expressed in healthy vascular cells but also in atherosclerotic plaques and after vascular injury, indicating an important role in response to injury and inflammatory processes [5]. Although most of the published data suggest not only a pathophysiological but also a physiological role for thrombin receptors in regulation of cardiovascular function, mice deficient in PAR1 or PAR2 both exhibit normal cardiac function, blood pressure levels and vasomotor responses to acetylcholine and angiotensin II [8,9].

2. PAR1 and vascular function: role of the endothelium

Kawabata et al. investigated the vascular response to a PAR1-activating peptide (TFLLR) and found that this peptide caused only a small NO-dependent relaxation in precontracted rat aortic rings but a substantial contraction

Abbreviations: EDHF, endothelium-derived hyperpolarizing factor; ET, endothelin; NO, nitric oxide; NOS, nitric oxide synthase; PAR, protease-activated receptor; SLIGRL, Ser-Leu-Ile-Gly-Arg-Leu-amide; TFLLR, Thr-Phe-Leu-Leu-Arg-amide; TRAP, thrombin receptor-activating peptides; VSMC, vascular smooth muscle cell

* Corresponding author. Tel.: +41-1-255 5663; fax: +41-1-255 8747.

E-mail address: barton@usz.ch (M. Barton).

in quiescent rings that was further increased after nitric oxide synthase (NOS) inhibition. However, in the rat superior mesenteric artery PAR1 causes only a weak contraction in quiescent rings that is substantially increased by endothelium removal or inhibition of NO- and EDHF-dependent pathways. Additionally, the authors provide evidence suggesting that superoxide does not play a role for these responses by using superoxide dismutase (SOD). In contrast to the findings by Kawabata et al. other studies demonstrated that thrombin and TRAPs cause a substantial endothelium-dependent relaxation of the aorta and coronary artery from different species [10–14]; these effects appear to be mainly mediated by cyclooxygenase products and NO [11,14]. Interestingly, after removal of the endothelium thrombin caused contractile responses in coronary arteries as observed in the present study [11,14], which may be due to PAR1-linked increases in vascular smooth muscle cell Ca^{2+} flux [15,16]. Additionally, contractile responses after PAR1 activation have been described even in the presence of an intact endothelium in human umbilical and placental arteries [17], in line with Kawabata et al. Taken together, these findings suggest that PAR1-mediated release of endothelium-derived contracting factors (EDCFs) may at least in part be responsible for the observed vascular contractions.

So what is the nature of these EDCFs? It has been shown that the endothelium produces endothelin-1 (ET-1), prostaglandin H_2 /thromboxane A_2 , and superoxide anion under certain conditions in response to other vasoactive substances [1,18]. Although the authors demonstrated that superoxide anion is not involved in the contractile responses observed, a possible role for prostaglandin H_2 /thromboxane A_2 or ET-1 was not investigated. Indeed, this has been demonstrated for PAR1 in the renal artery [19] and for PAR2 as discussed below [20,21]. It is noteworthy that thromboxane A_2 and its receptor have been shown to mediate thrombin-induced contractions in endothelium-denuded human arteries [22]. Moreover, thrombin also facilitates production and release of ET-1 [23,24], which could also explain the PAR1-mediated contractions observed by Kawabata et al. The fact that PAR1 is activated at low thrombin concentrations suggests a pathophysiological role for this receptor based on the observations made in these experimental studies. The data discussed above indicate functional differences of PAR1 on endothelial cells and VSMCs depending on the vascular bed investigated. Furthermore, it has been shown that agonists of PAR1 cause a biphasic vascular response, including both relaxation and contraction [19].

3. A link between PAR2 and EDHF?

Trypsin and PAR2-activating peptides can induce endothelium-dependent relaxation in rat [25] and rabbit aorta [20] as well as in other arteries [26–29], which is mainly due to NO. Kawabata et al. also evaluated the role of PAR2

for endothelium-dependent vasoreactivity in isolated rat mesenteric artery and aorta by using SLIGRL and trypsin. The results of these experiments show that these agonists lead to a substantial NO-dependent relaxation in rat aorta and cause complete endothelium-dependent relaxation in rat mesenteric arteries that is only in part inhibited by N^G -nitro-L-arginine methyl ester (L-NAME, a nonselective inhibitor of NOS) and indomethacin (a nonselective inhibitor of cyclooxygenase). Thus, the authors also present evidence that another endothelium-dependent principle is involved in PAR2-mediated signaling in the vascular wall of resistance arteries. Kawabata et al. demonstrate that the vasodilatory effect in response to PAR2 activation involves EDHF and gap-junction signaling. These observations could be explained by the caliber of resistance arteries, in line with previous data showing that the contribution of EDHF for endothelium-dependent relaxation increases with decreasing vessel diameter [30]. Similar observations were made in mice with atherosclerosis [31], and EDHF may contribute to abnormal endothelial vasoreactivity in patients with atherosclerosis [32]. Kawabata et al. elegantly demonstrate a role for EDHF in the PAR2-mediated vasorelaxation in the mesenteric artery by blocking small-, intermediate- and large-conductance Ca^{2+} -sensitive K^+ channels. The authors also show involvement of gap junctions since the relaxant response was blocked by carbenoxolone, an inhibitor of gap junctional intercellular communication. The findings of Kawabata et al. are in agreement with a recent study suggesting a role for EDHF in PAR2-mediated vasodilation of coronary arteries [33] that may also be functional in other vascular beds such as the gastric artery [34].

PAR2-activation did not cause contractions in rat mesenteric artery rings as reported by Kawabata et al., although PAR2-mediated vasoconstriction has been observed in different vascular beds and appears to be both endothelium dependent [20,21] as well as endothelium independent [35]. This endothelium-dependent vasoconstrictor response is possibly due to a yet unidentified contractile factor (EDCF) from human endothelial cells and may be mediated via a novel PAR2 receptor subtype, at least in human umbilical veins [21].

The findings by Kawabata et al. demonstrate a distinct role for EDHF and gap junction signaling in PAR2-mediated vascular responses in mesenteric resistance arteries. Whether these mechanisms are important for maintaining vascular integrity in diseases such as atherosclerosis [32], arterial thrombosis, or restenosis remains to be determined.

Acknowledgements

The authors work is supported by the Swiss National Foundation (SCORE 32.58421.99 and 32-58426.99/1), the Kurt und Senta Hermann Stiftung, and the Hanne Liebermann Stiftung, Zürich.

References

- [1] Traupe T, Ortmann J, Münter K, Barton M. Endothelial therapy of atherosclerosis and its risk factors. *Curr Vasc Pharmacol* 2003;9: 111–21.
- [2] Harrison DG. Cellular and molecular mechanisms of endothelial cell dysfunction. *J Clin Invest* 1997;9:2153–7.
- [3] Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, Weston AH. EDHF: bringing the concepts together. *Trends Pharmacol Sci* 2002;8:374–80.
- [4] Luscher TF, Barton M. Biology of the endothelium. *Clin Cardiol* 1997;8(Suppl. II):II-3–10.
- [5] Patterson C, Stouffer GA, Madamanchi N, Runge MS. New tricks for old dogs: nonthrombotic effects of thrombin in vessel wall biology. *Circ Res* 2001;10:987–97.
- [6] Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. Proteinase-activated receptors. *Pharmacol Rev* 2001;2:245–82.
- [7] Vu TK, Hung DT, Wheaton VI, Coughlin SR. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* 1991;6:1057–68.
- [8] Darrow AL, Fung-Leung WP, Ye RD, et al. Biological consequences of thrombin receptor deficiency in mice. *Thromb Haemost* 1996;6: 860–6.
- [9] Damiano BP, Cheung WM, Santulli RJ, et al. Cardiovascular responses mediated by protease-activated receptor-2 (PAR-2) and thrombin receptor (PAR-1) are distinguished in mice deficient in PAR-2 or PAR-1. *J Pharmacol Exp Ther* 1999;2:671–8.
- [10] Muramatsu I, Laniyonu A, Moore GJ, Hollenberg MD. Vascular actions of thrombin receptor peptide. *Can J Physiol Pharmacol* 1992;7:996–1003.
- [11] Ku DD, Zaleski JK. Receptor mechanism of thrombin-induced endothelium-dependent and endothelium-independent coronary vascular effects in dogs. *J Cardiovasc Pharmacol* 1993;4:609–16.
- [12] Tesfamariam B. Thrombin receptor-mediated vascular relaxation differentiated by a receptor antagonist and desensitization. *Am J Physiol* 1994;5 Pt 2:H1962–7.
- [13] Tesfamariam B. Distinct receptors and signaling pathways in alpha-thrombin- and thrombin receptor peptide-induced vascular contractions. *Circ Res* 1994;5:930–6.
- [14] Ku DD, Dai J. Expression of thrombin receptors in human atherosclerotic coronary arteries leads to an exaggerated vasoconstrictory response in vitro. *J Cardiovasc Pharmacol* 1997;5:649–57.
- [15] Antonaccio MJ, Normandin D, Serafino R, Moreland S. Effects of thrombin and thrombin receptor activating peptides on rat aortic vascular smooth muscle. *J Pharmacol Exp Ther* 1993;1:125–32.
- [16] Antonaccio MJ, Normandin D. Role of Ca^{2+} in the vascular contraction caused by a thrombin receptor activating peptide. *Eur J Pharmacol* 1994;1:37–44.
- [17] Tay-Uyboco J, Poon MC, Ahmad S, Hollenberg MD. Contractile actions of thrombin receptor-derived polypeptides in human umbilical and placental vasculature: evidence for distinct receptor systems. *Br J Pharmacol* 1995;4:569–78.
- [18] Barton M, Traupe T, Haudenschild CC. Endothelin, hypercholesterolemia and atherosclerosis. *Coron Artery Dis* 2003;7:477–90.
- [19] Derkach DN, Ihara E, Hirano K, Nishimura J, Takahashi S, Kanaide H. Thrombin causes endothelium-dependent biphasic regulation of vascular tone in the porcine renal interlobar artery. *Br J Pharmacol* 2000;8:1635–42.
- [20] Roy SS, Saifeddine M, Loutzenhiser R, Triggie CR, Hollenberg MD. Dual endothelium-dependent vascular activities of proteinase-activated receptor-2-activating peptides: evidence for receptor heterogeneity. *Br J Pharmacol* 1998;7:1434–40.
- [21] Saifeddine M, Roy SS, Al-Ani B, Triggie CR, Hollenberg MD. Endothelium-dependent contractile actions of proteinase-activated receptor-2-activating peptides in human umbilical vein: release of a contracting factor via a novel receptor. *Br J Pharmacol* 1998;7: 1445–54.
- [22] Yang Z, Arnet U, Bauer E, et al. Thrombin-induced endothelium-dependent inhibition and direct activation of platelet-vessel wall interaction. Role of prostacyclin, nitric oxide, and thromboxane A₂. *Circulation* 1994;5:2266–72.
- [23] Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;616:411–5.
- [24] Kohno M, Yokokawa K, Horio T, et al. Release mechanism of endothelin-1 and big endothelin-1 after stimulation with thrombin in cultured porcine endothelial cells. *J Vasc Res* 1992;2:56–63.
- [25] al-Ani B, Saifeddine M, Hollenberg MD. Detection of functional receptors for the proteinase-activated-receptor-2-activating polypeptide, SLIGRL-NH₂, in rat vascular and gastric smooth muscle. *Can J Physiol Pharmacol* 1995;8:1203–7.
- [26] Hwa JJ, Ghibaudi L, Williams P, et al. Evidence for the presence of a proteinase-activated receptor distinct from the thrombin receptor in vascular endothelial cells. *Circ Res* 1996;4:581–8.
- [27] Hamilton JR, Nguyen PB, Cocks TM. Atypical protease-activated receptor mediates endothelium-dependent relaxation of human coronary arteries. *Circ Res* 1998;12:1306–11.
- [28] Sobey CG, Cocks TM. Activation of protease-activated receptor-2 (PAR-2) elicits nitric oxide-dependent dilatation of the basilar artery in vivo. *Stroke* 1998;7:1439–44.
- [29] Sobey CG, Moffatt JD, Cocks TM. Evidence for selective effects of chronic hypertension on cerebral artery vasodilatation to protease-activated receptor-2 activation. *Stroke* 1999;9:1933–40 [discussion 1941].
- [30] Shimokawa H, Yasutake H, Fujii K, et al. The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. *J Cardiovasc Pharmacol* 1996;5:703–11.
- [31] d'Uscio LV, Barton M, Shaw S, Luscher TF. Chronic ET(A) receptor blockade prevents endothelial dysfunction of small arteries in apolipoprotein E-deficient mice. *Cardiovasc Res* 2002;2:487–95.
- [32] Fichtlscherer S, Dimmeler S, Breuer S, Busse R, Zeiher AM, Fleming I. Inhibition of cytochrome P450 2C9 improves endothelium-dependent, nitric oxide-mediated vasodilatation in patients with coronary artery disease. *Circulation* 2004;109:178–83.
- [33] McLean PG, Aston D, Sarkar D, Ahluwalia A. Protease-activated receptor-2 activation causes EDHF-like coronary vasodilation: selective preservation in ischemia/reperfusion injury: involvement of lipoxygenase products, VR1 receptors, and C-fibers. *Circ Res* 2002;4: 465–72.
- [34] Kawabata A, Nakaya Y, Kuroda R, et al. Involvement of EDHF in the hypotension and increased gastric mucosal blood flow caused by PAR-2 activation in rats. *Br J Pharmacol* 2003;2:247–54.
- [35] Komuro T, Miwa S, Minowa T, et al. The involvement of a novel mechanism distinct from the thrombin receptor in the vasoconstriction induced by trypsin. *Br J Pharmacol* 1997;5:851–6.